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(71) Applicant *(for all designated States except US)*: **THOMAS JEFFERSON UNIVERSITY** [US/US]; Office of Technology Transfer, 1020 Locust Street, Room M-60, Philadelphia, PA 19107-6799 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HOOPER, Douglas, C.** [CA/US]; 2 Oregon Trail, Medford, NJ 08055 (US). **DIETZSCHOLD, Bernhard** [US/US]; 3430 Goshen Road, Newtown Square, PA 19073 (US).

(74) Agent: **MONACO, Daniel, A.**; Drinker Biddle & Reath, LLP, One Logan Square, 18th & Cherry Streets, Philadelphia, PA 19103-6996 (US).

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(54) Title: RECOMBINANT ANTIBODIES, AND COMPOSITIONS AND METHODS FOR MAKING AND USING THE SAME

(57) Abstract: Recombinant antibodies are disclosed. The nucleic acid and encoded amino acid sequences of the heavy and light chain immunoglobulins of human monoclonal rabies virus neutralizing antibodies, and their use, are described.

rabies and rabies-related viruses associated with bats have recently been identified in the UK and Australia.

Rabies virus is characteristically bullet-shaped, enveloped particle of, on average, 75 by 180 nanometers. The virion consists of a single-stranded negative sense RNA genome and five structural proteins: the nucleoprotein (N) molecules, the phospho-protein (NS), the polymerase (L), the matrix protein (M) and the viral glycoprotein (G).

The N and G proteins both bear antigenic determinants which enable serotypic characterization of diverse rabies virus strains. N determinants are highly conserved between different virus isolates and are therefore very useful targets for the immunohistological detection of rabies virus infection using specific antibodies. On the other hand, antigenic determinants carried on the G-protein vary substantially among the rabies virus strains. Virus-neutralizing antibodies raised by vaccination with inactivated virus are directed against G. While it is clear that T cell responses to G, N, and NS, participate in immune responses to the virus under experimental conditions, assessment of immunity to rabies virus is generally limited to serology, particularly with respect to virus-neutralizing antibodies.

In areas of the world where human rabies is still common, the dog is the major reservoir of the viruses that infect man. Where canine rabies has largely been eliminated by vaccination, foxes, coyotes, skunks, raccoons, bats, and a variety of other mammals harbor variants of the virus. In many areas, wildlife reservoirs of virus continue to expand. Moreover, rabies virus can be transmitted from a reservoir species to humans or other end stage hosts by animals not normally associated with rabies, such as cats, rabbits, etc.

Almost invariably fatal once clinical symptoms appear, rabies can be averted by prompt treatment of an infected individual with a combination of passive and active immunization. Passive immunization consists of the administration of pre-formed rabies virus neutralizing antibodies obtained from pooled serum of rabies immune individuals (Human rabies-immune globulin; HRIG) or hyper-immunized horses (Equine rabies-immune globulin; ERIG). Both types of reagent present certain risks to recipients including variable antigen specificity, and thus potency, for different rabies virus isolates.

HRIG is prepared from pooled human sera, therefore there is the possibility

heterologous expression systems. These constructs allow rabies neutralizing human antibodies of defined specificity to be produced in a controlled system, purified away from possible deleterious contaminants. The present invention relates to these monoclonal rabies virus neutralizing human antibodies, the nucleic acid sequences of their heavy and light chains and the amino acid sequences of the encoded proteins. Also provided in the present invention are methods of using the monoclonal antibodies as a therapeutically effective post-exposure prophylactic treatment of individuals exposed to rabies virus.

SUMMARY OF THE INVENTION

The present invention provides recombinant antibodies, and compositions for and methods of producing such antibodies. According to some aspects of the invention, the present invention provides recombinant anti-rabies antibodies, and compositions for and methods of producing such antibodies. According to some aspects of the invention, the present invention provides recombinant antibodies with a specific constant region that makes them particularly effective in combating pathogens which attack the neural system.

The present invention further relates to isolated DNA sequences, to recombinant vectors comprising such sequences, to host cells comprising such vectors and methods of producing recombinant antibodies using such host cells.

The present invention additionally relates to the use of recombinant antibodies in the diagnosis, prevention and treatment of pathogen infections of neuronal tissue, particularly rabies.

The present invention provides isolated nucleic acid molecules having a heavy chain and a light chain nucleic acid sequence encoding a heavy chain and a light chain amino acid sequence. The heavy chain and light chain amino acid sequences are that of a monoclonal rabies virus neutralizing antibody that specifically binds to a rabies virus protein.

The present invention provides isolated nucleic acid molecules that encode the monoclonal rabies virus neutralizing antibody are derived from cDNA sequences of the heavy chain SEQ. ID. NO:1 and the light chain SEQ. ID. NO:2.

The present invention provides an isolated human monoclonal rabies virus

to the glycoprotein of various rabies virus strains. Post-exposure treatment with monoclonal antibody, or a mixture of a variety of monoclonal antibodies, will neutralize the rabies virus at the site of entry and prevent the virus from spreading to the central nervous system (CNS). Thus, for transdermal or mucosal exposure to rabies virus, rabies specific-monoclonal antibodies are instilled into the bite site, as well as administered systemically. Since viral replication is restricted almost exclusively to neuronal cells, neutralization and clearance of the virus by the monoclonal antibodies of the present invention prior to entry into the CNS is an effective post-exposure prophylactic.

One aspect of the present invention provides sequences of monoclonal antibodies against rabies virus. While most of the variable region of MAb 57 is well known (Cheung et al., *J. Virol.* 66:6714-6720, 1992, which is incorporated herein by reference), the constant region is not. The entire monoclonal antibody, both constant and variable regions, has been cloned and sequenced. The present invention provides the novel nucleotide sequence of MAb 57 constant region, nucleotides 476-1431, which includes constant domain 1 (CH1) and the hinge region. This sequence may be used in recombinant antibodies including anti-rabies antibodies or recombinant antibodies directed at other pathogens which attack neuronal tissue, such as encephalitis or herpes.

The invention relates to the recombinant antibodies, to the clones genes that encode them, to the vectors which incorporate cloned genes and host cells that include the vectors. The invention also provides methods of making and using the recombinant antibodies.

The present invention provides recombinant antibodies derived from MAb 57. MAb 57 derived from hybridomas are IgG2 antibodies; recombinant antibodies derived from MAb 57 are IgG1 antibodies. The invention relates to the recombinant antibodies derived from Mab 57, to the clones genes that encode them, to the vectors which incorporate cloned genes and host cells that include the vectors. The invention also provides methods of making and using the recombinant antibodies.

The present invention also provides the entire sequence of the heavy and light chains of the anti-rabies monoclonal antibody MAb JA. The invention relates to the recombinant antibodies derived from Mab JA, to the clones genes that encode them, to the vectors which incorporate cloned genes and host cells that include the vectors. The

immobilized or entrapped cell culture, e.g. in hollow fibres, microcapsules, on agarose microbeads or ceramic cartridges.

Degenerated sequences are degenerated within the meaning of the genetic code in that an unlimited number of nucleotides are replaced by other nucleotides without resulting in a change of the amino acid sequence originally encoded. Such degenerated sequences may be useful due to their different restriction sites and/or frequency of particular codons which are preferred by the specific host, particularly E. coli, to obtain an optimal expression of the recombinant antibody.

Furthermore the invention concerns a recombinant DNA which is a hybrid vector comprising an insert coding for the recombinant antibody described hereinbefore, and, optionally an origin of replication or an autonomously replicating sequence, one or more dominant marker sequences, expression control sequences, signal sequences and additional restriction sites.

Vectors typically perform two functions in collaboration with compatible host cells. One function is to facilitate the cloning of the nucleic acid that encodes the immunoglobulin domains, i.e. to produce usable quantities of the nucleic acid (cloning vectors). The other function is to provide for replication and expression of the recombinant gene constructs in a suitable host, either by maintenance as an extrachromosomal element or by integration into the host chromosome (expression vectors). A cloning vector comprises the recombinant gene constructs as described above, an origin of replication or an autonomously replicating sequence, dominant marker sequences and, optionally, signal sequences and additional restriction sites. An expression vector additionally comprises expression control sequences essential for the transcription and translation of the recombinant genes.

An origin of replication or an autonomously replicating sequence is provided either by construction of the vector to include an exogeneous origin such as derived from Simian virus 40 (SV 40) or another viral source, or by the host cell chromosomal mechanisms.

The markers allow for selection of host cells which contain the vector. Selection markers include genes which confer resistance to heavy metals such as copper or to antibiotics such as geneticin (G-418) or hygromycin, or genes which complement a

induced by isopropyl-.beta.-D-thiogalactoside, the trp (tryptophan) promoter regulated by the trp repressor and induced e.g. by tryptophan starvation, and the tac (hybrid trp-lac promoter) regulated by the lac repressor.

Vectors which are suitable for replication and expression in yeast contain a yeast replication start and a selective genetic marker for yeast. One group of such vectors includes so-called ars sequences (autonomous replication sequences) as origin of replication. These vectors are retained extrachromosomally within the yeast cell after the transformation and are replicated autonomously. Furthermore, vectors which contain all or part of the 2μm plasmid DNA from Saccharomyces cerevisiae can be used. Such vectors will get integrated by recombination into 2μm plasmids already existing within the cell, or replicate autonomously. 2μm sequences are particularly suitable when high transformation frequency and high copy numbers are to be achieved.

Expression control sequences which are suitable for expression in yeast are, for example, those of highly expressed yeast genes. Thus, the promoters for the TRP1 gene, the ADHI or ADHII gene, acid phosphatase (PHO3 or PHO5) gene, isocytochrome gene or a promoter involved with the glycolytic pathway, such as the promoter of the enolase, glyceraldehyde-3-phosphate kinase (PGK), hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase and glucokinase genes, can be used.

Vectors suitable for replication and expression in mammalian cells are preferably provided with promoting sequences derived from DNA of viral origin, e.g. from Simian virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus 2, bovine papilloma virus (BPV), papova-virus BK mutant (BKV), or mouse or human cytomegalovirus (CMV). Alternatively, the vectors may comprise promoters from mammalian expression products, such as actin, collagen, myosin etc., or the native promoter and control sequences which are normally associated with the desired gene sequence, i.e. the immunoglobulin H-chain or L-chain promoter.

Some preferred vectors are suitable for both procaryotic and eucaryotic hosts and are based on viral replication systems. Particularly preferred are vectors comprising Simian virus promoters, e.g. pSVgpt or pSVneo, further comprising an

with a hybrid vector according to the invention, and the transformed cells are selected. Transformation of microorganisms is carried out as described in the literature, for example for S. cerevisiae (A. Hinnen et al., Proc. Natl. Acad. Sci. USA 75: 1929, 1978), for B. subtilis (Anagnostopoulos et al., J. Bacteriol. 81: 741, 1961), and for E. coli (M. Mandel et al., J. Mol. Biol. 53: 159, 1970).

Accordingly, the transformation procedure of E. coli cells includes, for example, Ca^{2+} pretreatment of the cells so as to allow DNA uptake, and incubation with the hybrid vector. The subsequent selection of the transformed cells can be achieved, for example, by transferring the cells to a selective growth medium which allows separation of the transformed cells from the parent cells dependent on the nature of the marker sequence of the vector DNA. Preferably, a growth medium is used which does not allow growth of cells which do not contain the vector. The transformation of yeast comprises, for example, steps of enzymatic removal of the yeast cell wall by means of glucosidases, treatment of the obtained spheroplasts with the vector in the presence of polyethylene glycol and Ca^{2+} ions, and regeneration of the cell wall by embedding the spheroplasts into agar. Preferably, the regeneration agar is prepared in a way to allow regeneration and selection of the transformed cells as described above at the same time.

Transformation of cells of higher eucaryotic origin, such as mammalian cell lines, is preferably achieved by transfection. Transfection is carried out by conventional techniques, such as calcium phosphate precipitation, microinjection, protoplast fusion, electroporation, i.e. introduction of DNA by a short electrical pulse which transiently increases the permeability of the cell membrane, or in the presence of helper compounds such as diethylaminoethyldextran, dimethyl sulfoxide, glycerol or polyethylene glycol, and the like. After the transfection procedure, transfected cells are identified and selected, for example, by cultivation in a selective medium chosen depending on the nature of the selection marker, for example standard culture media such as Dulbecco's modified Eagle medium (DMEM), minimum essential medium, RPMI 1640 medium and the like, containing e.g. the corresponding antibiotic.

The recombinant antibodies according to the invention can be used for the qualitative and quantitative determination of the presence of rabies virus. In general, the recombinant antibodies according to the invention can be used in any of the known

For immunostaining cryosections of cryopreserved biopsy material or paraffin embedded tissue sections are treated with a solution containing a recombinant antibody of the invention comprising a detectable enzyme. Bound recombinant antibody is detected by treatment with a suitable enzyme substrate, preferably an enzyme substrate which leads to a solid deposit (stain) at the site of the recombinant antibody of the invention. In place of recombinant antibodies comprising an enzyme, a recombinant antibody comprising streptavidin and a solution of a biotin-enzyme-conjugate may be used, which leads to higher enzyme concentration at the site of the antibody and hence increased sensitivity of the immunostaining method. The solid deposit of the enzyme substrate is detected by inspection with a microscope, for example with a fluorescence microscope, or by scanning the optical density at the wavelength of the stain.

The use according to the invention of recombinant antibodies as described hereinbefore for the determination of rabies virus also includes other immunoassays known per se, for example immunofluorescence assays, latex agglutination with antibody-coated or antigen coated latex particles, hemagglutination with antibody-coated or antigen-coated red blood corpuscles, evanescent light assays using an antibody-coated optical fibre and other direct-acting immunosensors which convert the binding event into an electrical or optical signal, or the like.

The invention also concerns test kits for the qualitative and quantitative determination of presence of rabies virus comprising recombinant antibodies of the invention and, optionally, adjuncts, positive and/or negative controls, buffers, instructions and descriptions of exemplary results.

Furthermore, the recombinant antibodies of the invention, in are useful for the prevention of rabies infection in patients suspected of possible exposure to rabies virus or the treatment of patients who have been infected with rabies.

The invention therefore also concerns pharmaceutical compositions comprising a therapeutically effective amount of a recombinant antibody according to the invention and a pharmaceutically acceptable carrier. Preferred are pharmaceutical compositions for parenteral application. Compositions for intramuscular, subcutaneous or intravenous application are e.g. isotonic aqueous solutions or suspensions, optionally prepared shortly before use from lyophilized or concentrated preparations. Suspensions in

were negative in tests for HIV and hepatitis B. The mouse-human hybrid heteromyeloma SHM-D33 cells utilized as hybridoma fusion partners (Teng, N.N. et al., *Proc. Natl. Acad. Sci. USA* 80, 7308, 1983) and B95-8 Epstein-Barr Virus (EBV)-transformed marmoset leukocytes used as a source of EBV (Henderson et al., *J. Exp. Med.* Vol 76, p. 152, 1977) were obtained from ATCC (Rockville, MD).

Rabies viruses

To assess the capacity of antibody preparations to neutralize a variety of rabies virus strains, a number of antigenically distinct fixed, laboratory strains, as well as two representative street rabies viruses, were used. Evelyn-Rokitnicki-Abelseth (ERA), challenge virus standard, either mouse brain adapted (CVS-24) or cell culture adapted (CVS-11), and Pitman-Moore (PM) fixed strains were obtained from the Thomas Jefferson University virus collection. Silver-haired bat rabies virus (SHBRV), which has been associated with most of the recent rabies cases in the United States of America, and coyote street rabies virus/Mexican dog rabies virus (COSRV), which is a member of the dog rabies viruses, were obtained as described (Morimoto et al., *Proc. Natl. Acad. Sci. USA*, Vol. 93, p. 5653, 1996). Virus purification and preparation of glycoprotein (G) and nucleoprotein (N) have been described elsewhere (Dietzschold et al., *World Health Organization*, Geneva, p. 175, 1996).

EBV-transformation of human PBLs

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) as detailed elsewhere (Plebanski et al., Immunology Vol. 75, p. 86, 1992). T cells were then depleted by negative selection using monoclonal anti-CD2 antibody-coated magnetic beads (Dynal Inc., Lake Success NY) and a magnetic particle concentrator (Dynal). CD-2-negative cells, primarily B cells, were collected and immortalized as previously described (Swaminathan, 1992). Briefly, B95-8 cells, cultured to confluency in $RPMI_{1640}$ (Gibco BRL Life Technologies, Grand Island NY) supplemented with 10% fetal bovine serum (FBS; Gibco), were lysed by freeze-thawing on dry ice to release intracellular EBV. Supernatant containing EBV was clarified by spinning at 1000 RPM for 10 min and by filtration through a 0.45μm filter. Virus was concentrated by centrifugation at 8000 RPM for 2 h at 4°C. 7×10^6 B cells (suspended in

minimum of three times by limiting dilution in microtiter plates. Cells were titrated in 96 well round bottom plates in 2-fold dilutions starting from 4 cells per well. Cells from wells containing an average of 0.25 cells or less were expanded for the collection of supernatant and further analysis.

Analysis of rabies virus-specific antibodies in ELISA

Antibody specificity and isotype was assessed in solid phase ELISA. Plates (PolySorb™, Nunc) were coated at room temperature in a humidified chamber overnight with 5*g/ml rabies ERA virus, glycoprotein, or nucleoprotein diluted in phosphate-buffered saline (PBS). The plates were then blocked with 5% powdered milk in PBS and washed in PBS containing 0.05% $Tween_{20}$ (PBS-Tween) prior to the addition of supernatant samples.

Following incubation at room temperature for 2 h, the plates were washed with PBS-Tween to remove unbound primary antibody and various enzyme-conjugated or biotinylated secondary antibodies specific for the various human heavy chain isotypes were added for 1 h at room temperature. Secondary antibody was detected either by the production of a soluble end product in the medium upon addition of the appropriate substrate (3,3',5,5'-tetramethylbenzidine (TMB) in phosphate-citrate buffer, or p-nitrophenyl phosphate (PNPP) in 0.1Mglycine buffer, Sigma) or following the addition of avidin-alkaline phosphatase (30 min at RT) and PNPP substrate. The peroxidase-TMB reaction was stopped bye the addition of 2M H_2SO_4. Absorbance values were read in a microplate spectrophotometer (Biotek, Winooski VT) at 450 nm for the TMB product and at 405 nm for the PNPP reaction.

RFFIT

Supernatant samples from each transformed cell line were assayed for the presence of rabies virus-neutralizing antibodies using a variation of the rapid fluorescent focus inhibition test (RFFIT) as previously described (Hooper, ASM Press, WA p. 1997). Supernatant samples (50 µl) were diluted in 96 well flat-bottom plates (Nune). Rabies virus dilution known to cause 80-90% infection of the indicator cells were added to each test sample, and the plates incubated at 37°C for 1 h. Negative media and positive rabies-immune serum control samples were included in each assay. After incubation, 30ul of a 1.8 x 10^6 cells/ml concentration of baby hamster kidney (BHK) cells were added to each

albumin (BSA) protein standards were included in each assay. After incubation, samples were read in a spectrophotometer at 595nm. Protein concentrations of test samples were calculated with reference to the absorbance of the BSA standards. The purity of all antibody preparations was assessed by electrophoresis in 12.5% polyacrylamide gel under reducing conditions (SDS-PAGE). Purified antibodies showed two major bands on SDS-PAGE corresponding to isolated heavy and light immunoglobulin chains.

Generation, isolation and sequencing of cDNA clones

Total RNA was isolated from JA hybridoma cell by using RNAzol B (Biotecx Laboratories, Houston). Reverse transcriptase reactions were performed at 42°C for 1 hr with avian myeloblastosis virus reverse transcriptase (Promega) and oligo(dT) primer. A portion of the reverse transcriptase products were subjected to polymerase chain reaction (PCR) amplification using heavy chain specific primers: IgG-HF1 primer (5'-ACCATGGAGTTTGGGCTGAG-3' (SEQ. ID.NO: 5), start codon; underline, accession # Y14737), and IgG-HR2 primer (5'-ACTCATTTACCCGGGGACAG-3' (SEQ. ID. NO: 6), stop codon; underline, accession # Y14737) or light chain specific primers: IgG-LF5 primer (5'-AGCATGGAAGCCCCAGCTCA-3' (SEQ. ID. NO: 7), start codon; underline, accession # M63438), and IgG-LR2 primer (5'-CTCTAACAGTCTCCCCTGTTG-3' (SEQ. ID. NO: 8), stop codon, underline accession # M63438). Amplification was carried out for 35 cycles of denaturation at 94°C for 60 seconds, annealing at 50°C for 60 seconds, and polymerization at 72°C for 90 seconds with Taq DNA polymerase (Promega). The PCR products (1.4 kb for heavy chain, .7 kb for light chain) were purified and sequenced by using the AmpliTaq cycle sequencing kit (Perkin-Elmer) with the specific primers. The PCR products were cloned into TA cloning vector, pCR2.1 (Invitrogen). The cloned heavy chain and light chain cDNA was sequenced by using the AmpliTaq cycle sequencing kit (Perkin-Elmer) with the specific primers.

Monoclonal rabies virus neutralizing antibody coding sequences

Monoclonal antibody cDNA, and sequences complementary thereto, are monoclonal antibody nucleic acids provided by the present invention. In a specific embodiment herein, a monoclonal antibody cDNA sequence is provided for the heavy chain (SEQ. ID. NO:1) and the light chain (SEQ. ID. NO:2) of the monoclonal antibody from clone JA, thus lacking any introns.

Sci. USA 85, 2444-2448, 1988. Chimerized antibodies have constant regions derived substantially or exclusively from human antibody constant regions and variable regions derived substantially or exclusively from the sequence of the variable region of a monoclonal antibody from each stable heterohybridoma (Champion, J.M., et al., *Journal of Immunological Methods*, 235 81-90, 2000).

Single chain antibodies or Fv fragments are polypeptides that consist of the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or without an interconnecting linker. Thus, the Fv comprises the entire antibody combining site.

Functional equivalents further include fragments of antibodies that have the same, or substantially the same, binding characteristics to those of the whole antibody. Such fragments may contain one or both Fab fragments of the F(ab').sub.2 fragment. Preferably the antibody fragments contain all the s*complement of determining region of the whole antibody, although fragments containing fewer than all of such regions, such as three, four or five complement determining regions, are also functional. The functional equivalents are members of the IgG immunoglobulin class and subclasses thereof, but may be or may combine any one of the following immunoglobulin classes: IgM, IgA, IgD, or IgE, and subclasses thereof. Heavy chains of various subclasses, such as the IgG subclasses, are responsible for different effector functions and thus, by choosing the desired heavy chain constant region, chimeric antibodies with desired effector function are produced. Preferred constant regions are gamma 1 (IgG1), gamma 3 (IgG3) and gamma 4 (IgG4). The light chain constant region can be of the kappa or lambda type.

The immunoglobulins of the present invention can be monovalent, divalent or polyvalent. Monovalent immunoglobulins are dimers (HL) formed of a chimeric heavy chain associated through disulfide bridges with a chimeric light chain. Divalent immunoglobulins are tetramers (H_2 L_2) formed of two dimers associated through at least one disulfide bridge.

Standard recombinant DNA techniques

Standard recombinant DNA techniques are described in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and by Ausubel et al.(Eds) "Current Protocols in Molecular Biology," Green Publishing

The vector into which the monoclonal antibody cDNA is spliced may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include, but are not limited to, plasmids from *E. coli*, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include, but are not limited to, derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages.

The vector containing the monoclonal antibody cDNA to be expressed is transfected into a suitable host cell, as described *infra*. The host cell is maintained in an appropriate culture medium, and subjected to conditions under which the cells and the vector replicate.

Chimeric antibodies

In general, the chimeric antibodies are produced by preparing, for each of the light and heavy chain components of the chimeric immunoglobulin, a fused gene comprising a first DNA segment that encodes at least the functional portion of the human rabies virus specific neutralizing, preferably glycoprotein, human variable region linked (e.g., functionally rearranged variable region with joining segment) to a second DNA segment encoding at least a part of human constant region. Each fused gene is assembled in or inserted into an expression vector. Recipient cells capable of expressing the gene products are then transfected with the genes. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulins or immunoglobulin chains are recovered.

Genes encoding the variable region of immunoglobulin heavy and light chains are obtained from lymphoid cells that produce the monoclonal rabies virus neutralizing antibodies. For example, the heterohybridoma cell lines that produce monoclonal antibody against the rabies glycoprotein provide a source of immunoglobulin variable region for the present chimeric antibodies. Constant regions are obtained from human antibody-producing cells by standard cloning techniques. Alternatively, because genes are representing the two classes of light chains an the give classes of heavy chains have been cloned, constant regions of human origin are readily available from these clones. Chimeric antibody binding fragments such as F(ab').sub.2 and Fab fragments are prepared by designing a chimeric heavy chain gene in truncated form. For example, a

functionally equivalent, gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within a heavy or light chain sequence which result in a silent change, thus producing a functionally equivalent monoclonal antibody.

In accordance with the present invention, nucleotide sequences coding for heavy and light chains of the monoclonal rabies virus neutralizing antibody, a fragment or analog thereof, are inserted into an appropriate expression vector. This vector which contains the necessary elements for transcription and translation of the inserted protein-coding sequence so as to generate recombinant DNA molecules that direct the expression of heavy and light chain immunoglobulins for the formation of monoclonal rabies virus neutralizing antibody.

The preferred recipient cell line is a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes. Further, they possess the mechanism for glycosylation of the immunoglobulin. A particularly preferred recipient cell is a myeloma cell line that does not produce immunoglobulin, such as Sp2/0. These cell lines produce only the immunoglobulin encoded by the transfected immunoglobulin genes. Myeloma cells can be grown in culture or in the peritoneum of mice where secreted immunoglobulin can be obtained from ascites fluid. Other lymphoid cells such as B lymphocytes or hybridoma cells can serve as suitable recipient cells.

Several methods exist for transfecting lymphoid cells with vectors containing immunoglobulin encoding genes. A preferred way of introducing DNA into lymphoid cells is by electroporation. In this procedure recipient cells are subjected to an electric pulse in the presence of the DNA to be incorporated. Another way to introduce DNA is by protoplast fusion. In this method, lysozyme is used to strip cell walls from bacteria harboring the recombinant plasmid containing the immunoglobulin gene. The resulting spheroplasts are fused with myeloma cells with polyethylene glycol. After protoplast fusion, the transfectants are selected and isolated. Another technique that can be used to introduce DNA into may cell types is calcium phosphate precipitation.

The immunoglobulin genes can also be expressed in nonlymphoid cells, such as bacteria or yeast. When expressed in bacteria, the immunoglobulin heavy chains

CLAIMS

1. An isolated nucleic acid molecule comprising one or more nucleic acid sequences that encode an amino acid selected from the group consisting of: SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

2. An isolated nucleic acid molecule of claim 1 comprising one or more nucleic acid sequences selected from the group consisting of: SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and SEQ ID NO:15.

3. A recombinant expression vector comprising a nucleic acid molecule of claim 2.

4. A host cell comprising an expression vector of claim 3.

5. A method of producing an isolated recombinant antibody comprising culturing a host cell of claim 4 and isolating recombinant antibodies expressed thereby.

6. A recombinant expression vector comprising a nucleic acid molecule of claim 1.

7. A host cell comprising an expression vector of claim 6.

8. A method of producing an isolated recombinant antibody comprising culturing a host cell of claim 7 and isolating recombinant antibodies expressed thereby.

9. A recombinant antibody comprising a constant region of Mab 57 linked to a non-MAb 57 variable region.

10. An isolated nucleic acid molecule that encodes the recombinant antibody of claim 9.

SEQUENCE LISTING

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Dietzschold, Bernhard

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ccagggaagg ggctggagtg ggtctcagct attagtgcta gtggtcatag cacatatttg 240
gcagactccg tgaagggccg gttcaccatc tccagagaca attccaagaa cacgctgtat 300
ctgcaaatga acagcctgag agccgaggac acggccgtat attactgtgc gaaagatcga 360
gaggttacta tgatagttgt acttaatgga ggctttgact actggggcca gggaacccgg 420
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His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
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Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
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Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Phe
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Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
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SEQ ID NO:10

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